GENETIC REGULATION OF A CONSTITUTIVE OPERON IN E. COLI B/r

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ABSTRACT

Complementation analysis, using a regulatory mutant in the constitutive d-ribose operon of E. coli B/r, have shown that the genetic regulation of constitutive operon may follow a truly positive control mechanism whereby the expression of the operon requires an active constitutive initiation protein to allow the synthesis of the structural genes products.

INTRODUCTION

Genetic and biochemical analysis of the regulation of the d-ribose metabolism in E. coli B/r have shown a constitutive d-ribose operon composed of the structural genes of the d-ribose permease and the d-ribokinase which maps in a position closely linked to the leucine locus (1,2). Of the d-ribose negative mutants analyzed, two mutants, AB7 and AB36, were found to be defective in both the d-ribose permease and the d-ribokinase activities and to map at the extreme left of the d-ribokinase structural gene (2), representing a possibility of their being regulatory mutants for the d-ribose operon. In an attempt to determine the nature of these mutants, as operator, promotor, regulator or simply as polarity mutants in the d-ribokinase structural gene, we have performed merozygote complementation analysis using one of these mutants, AB36. In this report the results of this analysis are presented.

MATERIALS AND METHODS

Bacterial Strains: The bacterial strains used here were the following, an

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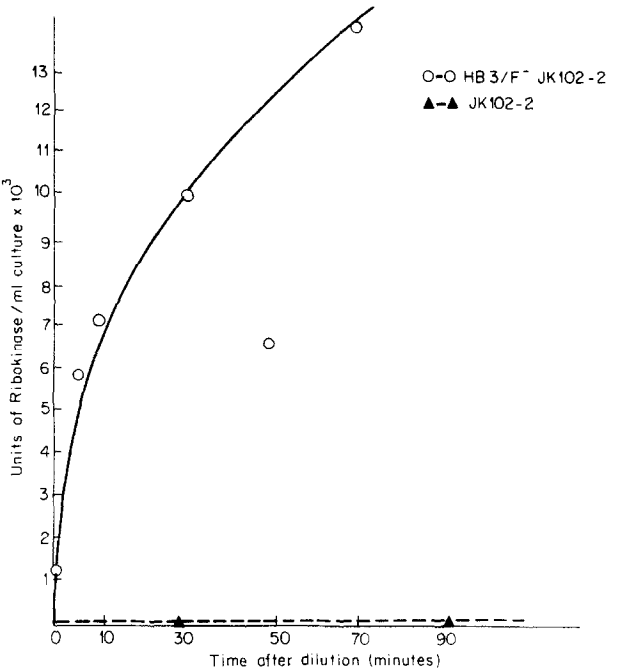

Figure 1: Kinetics of constitutive d-ribokinase synthesis in merzygotes of HB3/F⁻ JK102-2. Enzyme synthesis is followed at 0, 5, 10, 30, 50 and 70 minutes after the end of the conjugation step. Units of d-ribokinase per ml culture was determined as the μmoles of C^{14} ribose-5-phosphate produced per hour per ml of culture. Culture turbidities were uniformly adjusted in the preparation of the samples sonicates (Materials and Methods).

E. coli B/r Hfr (HB3) kindly donated by H. Boyer (3), E. coli B/r F⁻ JK102-2 a leucine negative d-ribokinase negative streptomycin resistant mutant and the d-ribokinase, d-ribose permease negative streptomycin resistant mutant, AB36, both previously described and mapped (2).

Complementation Analysis: Complementation analysis was performed using a procedure similar to that described by Helling and Weinberg (4) as follows: L-broth overnight cultures of HB3, transduced to rbs^+ by P_1 transduction, JK102-2 and AB36 were diluted in fresh L-broth to a turbidity of 80 Klett units (blue filter) and regrown for 1 hour on a gyrotory incubator shaker at 37°C. Conjugation mixtures, HB3/JK102-2 and HB3/AB36 were then made using an Hfr/F⁻ ratio of 1/5 in a total volume of 55 ml in 1 liter flasks. Conjugation was allowed for 40 minutes at 37°C at a 60 rpm shaking speed to allow for the transfer of the rbs^+ gene and the conjugation mixtures were

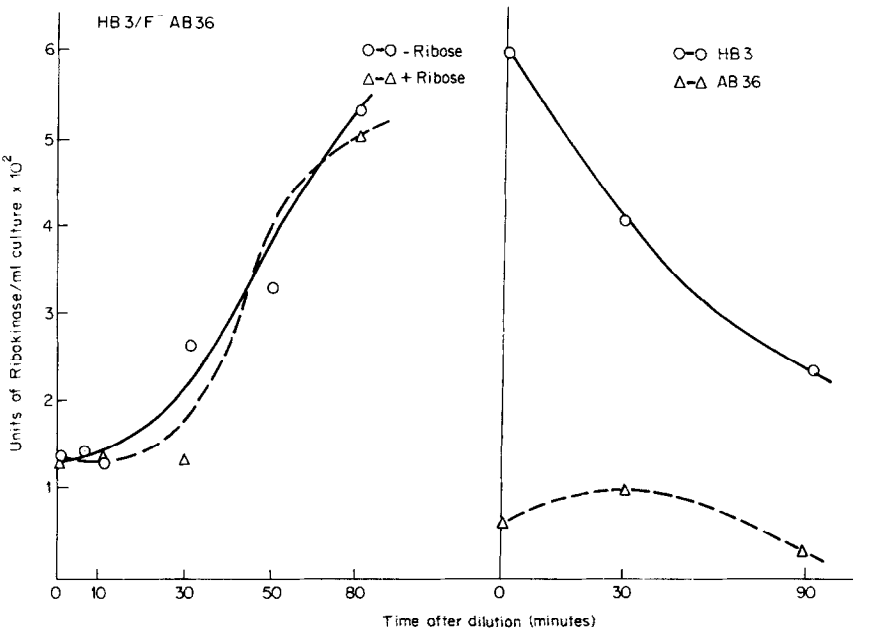

Figure 2: Kinetics of d-ribokinase synthesis in merozygotes of HB3/F⁻ AB36, in the presence and absence of d-ribose in the dilution medium. Enzyme synthesis was followed as described under Figure 1 and Materials and Methods.

immediately transferred to 2700 ml Fernbach flasks each containing 285 ml L-broth-streptomycin media. Aliquots of 45 ml were removed after vigorous shaking at time intervals and placed in centrifugation cups, containing 400 µg/ml chloramphenicol, chilled in ice baths and similarly 0.1 ml aliquots were plated on minimal media selective for *rbs*$^+$ recombinants. Five ml aliquots were removed from the various samples for turbidity determination and discarded. Samples were centrifuged under refrigeration at 6000 rpm for 15 minutes, and washed with EDTA (10^{-3} M, pH 7.6). The washed pellets were resuspended in EDTA-glutathion buffer (10^{-3} M, pH 7.6) and the buffer volume was adjusted according to the turbidity of the sample to maintain uniformly dense suspensions for even sonication. Two ml aliquots of each suspension were sonicated for 1 minute, cell debris then removed by centrifugation at 10,000 rpm for 30 minutes and the d-ribokinase determination was performed on the supernatants.

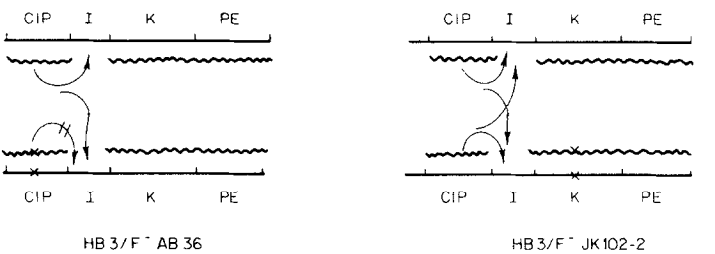

Figure 3: A possible model for the genetic regulation of the d-ribose constitutive operon. In merozygotes HB3/F⁻JK102-2, the d-ribokinase deficiency is due to a structural gene mutation in the rbsA (kinase) gene, immediate expression of the exogenote is allowed by the active CIP present in the cytoplasm of the recipient cells. In HB3/F⁻AB36, the lack of active CIP, is responsible for the d-ribose permease, d-ribokinase deficiencies of AB36. Expression of d-ribokinase structural genes of the endogenote and the exogenote, is thus dependent upon the synthesis of an active CIP product which is delayed until the introduction and the expression of the CIP (rbsC) gene of the exogenote.

Enzyme Assays: Determination of the d-ribokinase activity was performed as described previously (1).

RESULTS AND DISCUSSION

Kinetics of D-Ribokinase Synthesis in HB3/JK102-2 Merozygote: As shown in Figure 1, constitutive synthesis of the d-ribokinase was found to occur immediately after the introduction of the rbs^+ gene. No enzyme synthesis, however, was detectable with the recipient JK102-2 alone, indicating that all regulatory elements required for the constitutive expression of the rbs^+ genes were already present in the recipient cells, thus allowing immediate expression of the d-ribokinase structural gene of the exogenate.

Kinetics of D-Ribokinase Synthesis in HB3/AB36 Merozygote: As shown in Figure 2, expression of the exogenate was delayed as indicated by a 10 minute lag in the constitutive synthesis of the d-ribokinase. Addition of d-ribose (0.1%) to the dilution medium (L-broth-streptomycin medium) in a duplicate experiment did not induce the enzyme synthesis, in fact, the lag period seems to have increased (Fig. 2). Enzyme synthesis by the streptomycin sensitive Hfr and the recipient AB36, used as controls, showed a reduction in the d-ribokinase level of the Hfr due to the streptomycin in the dilution medium

and the basal level of activity of AB36 as expected (Fig. 2). $\underline{Rbs}^+$ recombinant formation in all three crosses, occurred at a 10^{-3}-10^{-4} frequency showing that conjugation has taken place.

Since the results with the HB3/AB36 complementation show a delay in the constitutive synthesis of the d-ribokinase of the merozygote in contrast to the immediate expression with JK102-2 it is indicated that a cytoplasmic product is involved in the regulation of the d-ribokinase gene expression, and thus the possibility of AB36 mutant being a promotor, operator or a polarity mutant are ruled out.

The question, therefore, is whether constitutive operons are regulated through an operator repressor interaction as is known for inducible and repressible systems. It is conceivable that in constitutive operons the repressor molecule has a low affinity for the operator gene, thus resulting in the constitutive expression of the operon. If so, a mutation in the regulator gene such as AB36 could result in an alteration in the repressor molecule increasing its recognition of the operator gene, thus turning off the constitutive expression of the operon. Although it should follow that the operon would become inducible, it is still possible that the repressor molecule of AB36 is a super repressor type such as in the I^S mutants of the lac operon (5) thus noninducible. Under these conditions, however, the experimental finding should be a complete turn off of the d-ribokinase synthesis in the HB3/AB36 merozygote, which was not found. The simplest model consistent with the above results, we feel, is as follows: a constitutive operon may be composed only of structural genes, an initiator or promotor gene, and a regulator gene coding for a constitutive initiation protein required for the initiation of the transcription process, possibly analogous to the catabolite gene activator protein (6) with no requirement for an operator gene. A mutational alteration in the regulator gene would therefore result in an inactive constitutive initiation protein and, thus, no transcription of the operon would occur. Introduction of wild type regulator

gene would, thus, allow the expression of the operon, after a lag period permitting the synthesis and accumulation of the constitutive initiation protein (Fig. 3). This model, to us, represents a truly positive control mechanism in which there is a definite requirement for an active initiator protein, not dependent upon its interaction with an extracellular inducer molecule. Consideration of other models requires more complicated mechanisms of incomplete dominance, subunit interactions and the like which would not be consistent with the nature of constitutive metabolic pathways. This model, of course, requires verification by reciprocal crosses and more importantly the isolation of more regulatory mutants, which is underway.

ACKNOWLEDGEMENTS

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Note added in proof. (HB3) should read (HB1) on p. 1016, lines 1 and 10; p. 1018, lines 4 and 11; p. 1019, line 4; and in figs. 1-3 and their respective legends.